Neuronal survival: **Early dependence on Schwann cells**

Alun M. Davies

Schwann cells fail to develop in mouse embryos lacking functional ErbB3 neuregulin receptors, and most sensory and motor neurons subsequently die in these mice. As ErbB3 acts cell autonomously in Schwann cell development but not in neuronal survival, neurons may depend on Schwann cells for survival.

Address: School of Biological and Medical Sciences, Bute Medical Buildings, University of St. Andrews, St. Andrews, Fife KY16 9TS, Scotland.
E-mail: amd2@st-and.ac.uk

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Neurons are produced in excess in the developing vertebrate nervous system, superfluous neurons being eliminated during a phase of cell death that occurs shortly after they start innervating their targets. Work on nerve growth factor (NGF) has led to the formulation of the neurotrophic hypothesis — the idea that developing neurons depend for their survival on a supply of a neurotrophic factor from the tissues they innervate. Classic studies of the effects of manipulating the availability of NGF to neurons in the developing nervous system — by administering exogenous NGF or function-blocking antibodies — demonstrated that the survival of sympathetic neurons and certain kinds of sensory neurons is dependent on NGF *in vivo*. Accordingly, these same neurons are eliminated in mice with targeted null mutations in either the gene for NGF or in the gene *trkA*, which codes for the NGF receptor tyrosine kinase. The finding that NGF is synthesized in the target tissues of NGF-dependent sensory and sympathetic neurons in proportion to their final innervation density, and that NGF synthesis commences with the arrival of the earliest nerve fibres at these targets, established that NGF really does act as a target-derived neurotrophic factor during development [1,2].

NGF is the founder member of a family of proteins termed the neurotrophins, which includes brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT3), NT4/5 and NT6. A wealth of *in vitro* and *in vivo* studies of the effects of these proteins on neurons, and studies of mice with targeted null mutations in genes encoding these proteins or their receptors, have extended the generality of the neurotrophic hypothesis [1–3]. In addition to the neurotrophins, several other proteins have been shown to promote the survival of developing neurons, including ciliary neurotrophic factor (CNTF), leukaemia inhibitory factor (LIF), glial-cell-line-derived growth factor (GDNF), neurturin and hepatocyte growth factor (HGF). Somewhat at variance with the neurotrophic hypothesis, however, recent studies of the *in vitro* survival requirements of neurons at different stages of development, and studies of the timing of neuronal death in mutant mice deficient in neurotrophins or their receptors, have shown that some neurons have a transient dependence on certain neurotrophins during the time that their axons are growing to their targets [4].

This transient neurotrophin dependence is, for example, exhibited by the trigeminal ganglion, a population of sensory neurons that innervates the face. Most of the neurons of the trigeminal ganglion are dependent on NGF derived from their cutaneous target tissues during the period of naturally occurring neuronal death. Earlier in development, however, when many axons are growing to their targets, most of these neurons can be supported in culture by BDNF or NT3, but not NGF. Accordingly, there is a marked decrease in the number of neurons in the trigeminal ganglia of *nt3*$^{-/-}$ and *trkB*$^{-/-}$ embryos (*trkB* encodes a receptor for BDNF and NT3) early in development, whereas increased loss of neurons in *trkA*$^{-/-}$ embryos occurs later in development, during the period of naturally occurring neuronal death [4].

Many neurons in early dorsal root ganglia also appear to be dependent on NT3 for survival before their axons reach their targets. There is a marked reduction in the number of neurons in the dorsal root ganglia of *nt3*$^{-/-}$ embryos during the early stages of ganglion formation, in part because of the death of postmitotic neurons. Furthermore, the expression of *nt3* mRNA in the tissues through which axons grow to reach their targets suggests that NT3 is available to dorsal root ganglion neurons before their axons reach their final targets [4].

This recent work has given rise to the idea that at least some neurons are dependent on intermediate trophic support derived from the cells that lie *en route* to their peripheral targets, before they become dependent on target-derived neurotrophins for survival (for detailed discussion, see [4]). The recent analysis [5] of mice that lack ErbB3, a neuregulin receptor, has provided compelling evidence that Schwann cells and/or their precursors, which are associated with peripheral axons along the course to their targets, are a major source of trophic support for early spinal motoneurons and dorsal root ganglion neurons.

The neuregulins are a group of polypeptide factors that play critical roles in the development of the nervous

system and heart, and in the morphogenesis of some epithelia [6,7]. These factors are derived by alternative RNA splicing from a single gene (*neuregulin-1*) and were separately identified as neu differentiation factor (NDF), heregulin, glial growth factor (GGF), acetylcholine receptor-inducing activity (ARIA) and sensory and motor neuron-derived factor (SMDF). Neuregulins are necessary for the development of the neurogenic lineage in cranial, but not trunk, neural crest. Neuronally derived neuregulins promote the survival of Schwann cell precursors, stimulate proliferation of mature Schwann cells and act at the neuromuscular junction to promote the end-stage differentiation of muscle cells. Neuregulins are also required for the formation of cardiac trabeculae, and defective heart development may be the reason why *neuregulin*$^{-/-}$ [8,9], *erbB2*$^{-/-}$ [10] and *erbB4*$^{-/-}$ [11] embryos die at mid-gestation.

Three members of the ErbB family of receptor tyrosine kinases — ErbB2, ErbB3 and ErbB4 — mediate the responses of cells to neuregulins [6,7]. Neuregulins bind to ErbB3 and ErbB4 with high affinity, and to ErbB2 with low affinity [12]. When ErbB2 is co-expressed with ErbB3 or ErbB4, however, neuregulin promotes tyrosine phosphorylation of ErbB2 as a result of receptor heterodimerization and transphosphorylation. Expression studies in cell lines have shown that in addition to ErbB2–ErbB3 heterodimers and ErbB2–ErbB4 heterodimers, ErbB4 homodimers are functional neuregulin receptors. ErbB3–ErbB3 homodimers, however, cannot signal neuregulin binding, because ErbB3 possesses very low or no kinase activity.

Mice that lack functional ErbB3 receptors have been generated by targeting mutations of the *erbB3* gene [5]. One of the most striking features of *erbB3*$^{-/-}$ embryos is the complete absence of Schwann cell precursors and Schwann cells in peripheral nerves. Although the histological appearance and number of cells in the dorsal root ganglia of ErbB3-deficient embryos is similar to that of wild-type embryos up to day 12.5, there is extensive apoptosis of neurons in dorsal root ganglia over the next few days of development, resulting in 70% fewer neurons by day 14.5 and 82% fewer by day 18.5. This suggests that dorsal root ganglion neurons differentiate and extend axons normally in the absence of ErbB3 receptors, but subsequently die at an early stage in their development. Spinal motoneurons also develop normally in ErbB3-deficient embryos but subsequently die (by day 18.5 the number of ventral root axons is reduced by 79% compared with wild-type embryos). The loss of motoneurons in ErbB3-deficient embryos takes place later in development than the loss of dorsal root ganglion neurons, and aspects of muscle innervation, such as the induction and clustering of acetylcholine receptors, have started to form normally.

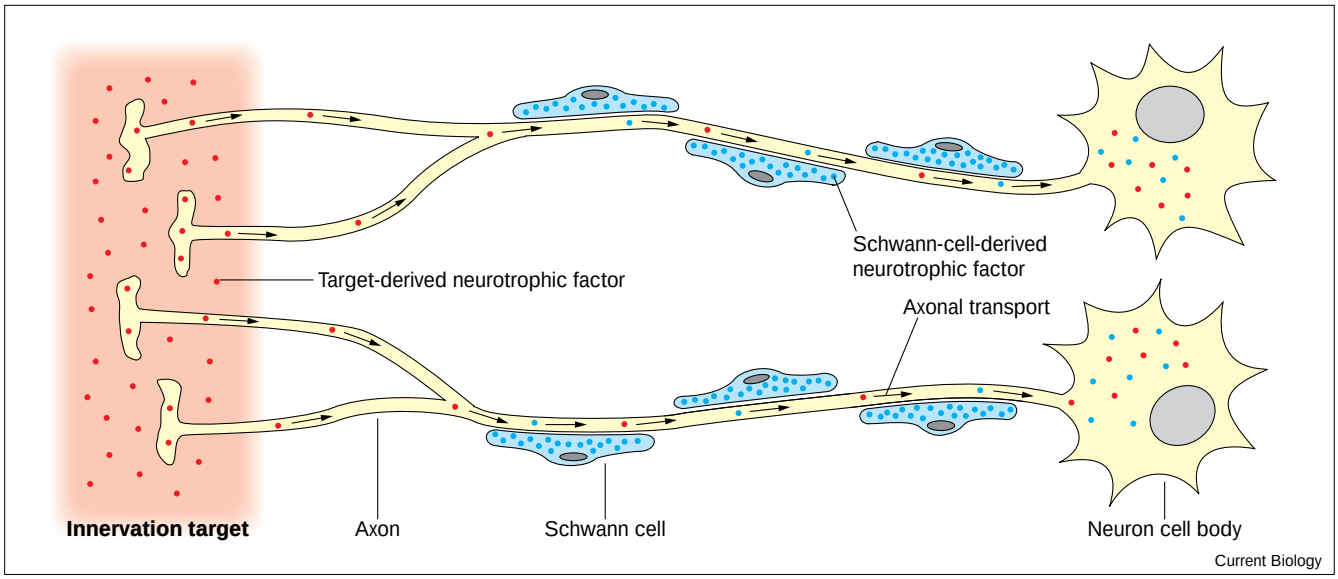
To determine whether the loss of spinal motor and sensory neurons in ErbB3-deficient embryos reflects a direct role of ErbB3 in mediating trophic signals in these neurons, or is a consequence of other changes — such as the loss of Schwann cell precursors — in the mutant embryos, *erbB3*$^{-/-}$ embryonic stem cells were injected into wild-type blastocysts to generate chimeric embryos [5]. Whereas the relative contributions of mutant and wild-type cells were similar in dorsal root ganglia and various other tissues in these chimeras, mutant cells made no contribution to Schwann cell precursors. This key experiment demonstrates that ErbB3 functions cell autonomously in the development of Schwann cells, but not in the survival of spinal motor and dorsal root ganglion neurons.

Because of the close association of Schwann cells and their precursors with developing peripheral axons, and because these cells are known to synthesize a variety of factors that are capable of promoting neuronal survival, the most parsimonious explanation for the loss of spinal motoneurons and dorsal root ganglion neurons in ErbB3-deficient embryos is the absence of trophic support from Schwann cells and/or their precursors, which fail to develop in these embryos. It is also possible, however, that these neurons may die as a consequence of other changes in the ErbB3-deficient embryos, such as decreased synthesis of, or impaired access to, target-derived neurotrophic factors.

The death of dorsal root ganglion neurons in ErbB3-deficient embryos at the stage when many axons are still growing towards their targets suggests that Schwann cells or their precursors provide intermediate trophic support to neurons whose axons have not yet reached their final targets. Schwann-cell-derived trophic support could also be important during the earliest stages of target field innervation, which appears to be the case for at least some motoneurons whose axons have reached their target muscles before most motoneurons die. Previous work has provided some evidence that at least some motoneurons are dependent on Schwann-cell-derived neurotrophic factors for survival in the adult. CNTF is expressed in Schwann cells and about a fifth of motoneurons are lost in adult *cntf*$^{-/-}$ mice [13]. Figure 1 shows the potential sources of trophic support, both target-derived and Schwann-cell-derived, for neurons of the peripheral nervous system.

One of the surprising aspects of the death of spinal motor and dorsal root ganglion neurons in ErbB3-deficient embryos is the scale of the loss. Far more neurons die than in any single neurotrophic factor knockout mouse so far described [1–3]. This could mean that Schwann cells and/or their precursors provide multiple neurotrophic factors that are required for the survival of early dorsal root ganglion neurons and motor neurons. Schwann cells have indeed been reported to synthesize a variety of neurotrophic

Figure 1



Neurons in the peripheral nervous system may obtain neurotrophic factors from their innervation targets and from Schwann cells or their precursors. Neurotrophic factors are conveyed to the neuronal cell body by retrograde axonal transport and promote survival.

factors including BDNF, NT3, CNTF, LIF, GDNF and HGF, all of which are capable of promoting the survival of sensory and motoneurons in culture to varying extents. Alternatively, Schwann cells may also produce other, as yet uncharacterized, neurotrophic factors, especially as no or relatively little death of motoneurons has been observed in embryos lacking each of the factors known to be produced by Schwann cells.

In *neuregulin-1*$^{-/-}$ and *erbB2*$^{-/-}$ embryos, neural-crest-derived cranial sensory neurons fail to differentiate, although populations of placode-derived sensory neurons are little affected in these embryos up to the time they die *in utero* (embryonic day 10.5) [8–10]. A very similar cranial phenotype is observed in *erbB3*$^{-/-}$ embryos at this early stage in development (C. Birchmeier, personal communication). Because *erbB3*$^{-/-}$ embryos survive longer than *neuregulin-1*$^{-/-}$ and *erbB2*$^{-/-}$ embryos [5], it will be informative to see whether, like dorsal root ganglion neurons, placode-derived sensory neurons undergo increased apoptosis at later stages. This is an interesting question because, unlike many neural-crest-derived sensory neurons that switch their neurotrophin requirements from NT3 or BDNF to NGF at an early stage in development when their axons are growing to their targets [4], placode-derived sensory neurons at a similar stage survive *in vitro* for varying lengths of time without neurotrophins. Placode-derived sensory neurons with more distant targets survive longer without neurotrophins than neurons with nearby targets [14]. Thus, it is possible that these populations of neurons are not dependent on intermediate support before encountering their targets, and may not be lost in large numbers in *erbB3*$^{-/-}$ embryos.

The generation and characterization of ErbB3-deficient mice have provided important insights into the potential interactions between neurons and Schwann cells that control neuronal survival in the developing nervous system. In particular, this work provides an especially compelling challenge to the widely held assumption that developing neurons are dependent solely on trophic support from their innervation targets in the developing peripheral nervous system. Elucidation of the particular Schwann-cell-derived factor(s) that appear to play such a prominent role in sustaining the survival of early spinal motor neurons and dorsal root ganglion neurons will be an important goal.

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